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Influence of soil pH in the effects of ZnONPs on the antioxidant activities and Zn uptake in three plant species (*T. aestivum*, *R. sativus* and *Z. mays*)

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In recent years, the study of phytotoxicity of NPs has made rapid progress, but important issues remain to be solved, among them, the role of soil and the importance of the physicochemical soil characteristics for their toxicity and accumulation potential. It is due to most available information about the NP toxicity in higher plants comes from studies on hydroponic media. Moreover, it is necessary to develop more sensitive and specific toxicity indicators based on biochemical changes for evaluating the toxicity of NPs. The ability to generate reactive oxygen species (ROS) has been identified as a possible mechanism of NP toxicity. ROS accumulation induces lipid peroxidation, membrane and DNA/RNA damage and

metabolism imbalance. Plants have evolved various protective mechanisms to limit oxidative damage caused by ROS accumulation such as the production of antioxidants (e.g. ascorbate, carotenoids or thiols) and antioxidant enzymes. The aims of this study were to study the influence of soil pH in ZnONP toxicity and accumulation to different plant species and to assist in the selection of potential biomarkers of soils contaminated with these nanoparticles. ZnONPs were selected because there is a growing interest in their use in agricultural formulations taking advantage of their properties as a UV blocker, or as fertilizers. The ZnO NP toxicity was tested in three plant species of agronomic interest: *Triticum aestivum*, *Rabanus sativus* and *Zea mays*. Two agricultural soils: an acid soil (pH 5.4) and a basic soil (pH 8.3) were spiked with 20, 225, 450 and 900 mg ZnO NP kg⁻¹ (Zn basis). The effects of the treated soils on ROS levels and the antioxidative defense system of plant species were analyzed after 35 days of exposure. Thus, the changes on the activity of the antioxidant enzymes (APX, GPOD and CAT), the levels of reduced glutathione and proteins, and the effects on lipid peroxidation were measured. To obtain a better understanding of the toxicology of ZnONPs, these effects were compared with visible damage at the functional level of the whole organism: seedling emergence and growth, and the changes in the chlorophyll levels. In addition, Zn concentration in roots and shoots of plant species were determined. This research was funded by the Spanish project RTA2013-00091-C02-01.